WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 1069-1078. Research Article ISSN 2277– 7105

SMALL MOLECULE ANTAGONIST FOR EGFR- A PRELIMINARY COMPUTATIONAL FRAMEWORK OF AZO DERIVATIVES AS THERAPEUTIC AGENT FOR BLADDER CANCER

K. Loganathan[1]*, K. Sithick Ali[1] , J. Gerad Rakesh[2] A. Mohan[3] , R. Thulasibabu[4], Rajani Gopal Gad[5] , M. Purushothaman[1] , S. Silambarasan[1], A. Jamal Abdul Nasser[1]

[1]Department of Chemistry, Jamal Mohamed College (Autonomous), Tiruchirappalli- 620020, India.

[2]Department of Microbiology, Sri Venkateshwaara Medical College Hospital and Research Centre, Ariyur, Puducherry – 605102

[3]Department of Animal Science, Bharathidasan University, Tiruchirappalli- 620024, India.

[4]Division of Molecular & Medical Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore – 641046, Tamil Nadu, India.

[5]Department of Biotechnology, SRM University, Kattankulathur-603203, Tamil Nadu, India.

Article Received on
20 May 2015,

Revised on 15 June 2015,
Accepted on 06 July 2015

***Correspondence for Author**
K. Loganathan
Department of Chemistry, Jamal Mohamed College (Autonomous), Tiruchirappalli- 620020, India.

ABSTRACT

Epidermal growth factor receptor (EGFR) is the well-established and iconic target for a variety of cancer types. The recent attractive molecular receptor identified is EGFR. Small molecule based drug discovery is the rapidly growing field wherein the design of therapeutics is the master approach till date. This study throws light on the series of azo derivatives from the one of our previous reports may evolve as the effective anti- EGFR agent. We worked with successful *insilico* approaches like molecular docking and pharmacokinetic properties. As a result, we found all the six azo derivatives are exceedingly capable of acting as antagonist for EGFR, further more we document that as molecule with therapeutic value in bladder cancer.

KEY WORDS: Bladder Cancer, EGFR, Glide, ADME.

INTRODUCTION

Bladder cancer is one of the leading cancers with estimated probable cases of 74,000 adults (56,320 men and 17,680 women) in the United States. It is estimated that 16,000 deaths (11,510 men and 4,490 women) from this disease will occur in 2015.[1] EGFR is trans

membrane protein, located at chromosome 7 that consists of 1186 amino acids mainly expressed on the surface of normal cells. The receptor has a molecular weight of 170KDa encoded by C-erbB-1proto-oncogene.[2,3] Based on the structure and function, the growth factor receptor is divided into four types - HER1/EGFR/C-erbB-1, HER2/C-erbB-2, HER3/C-erbB-3, and HER4/C-erbB-4.[4] The epidermal growth factor receptor, possessing angiogenic properties or activity, binds with number of endogenous ligands like EGFR belongs to the erbB family of closely related receptor tyrosine kinases, which include erbB1 (also known as EGFR), erbB2 (HER2), erbB3, and erbB4. The binding of EGF transforming growth factor-α with EGFR induces conformational catalytic changes within the receptor and increases the intrinsic tyrosine kinase activity resulting in auto phosphorylation of several tyrosine residues within the carboxyl terminal of the EGFR and which activate the various downstream signal transduction pathways.[5,6,7] EGFR is one of the attractive molecular drug targets in the design of small molecule inhibitors for the treatment of patients with mutant form of EGFR. Over expression of EGFR can lead to develop kidney and bladder cancer. The platform of drug designing research demonstrated different success stories with the help of novel computational methods. In this study, classical molecular docking featuring lock and key model is followed to figure out the binding preference of azo derivatives with the EGFR and pharmacokinetics studies also carried out to ascertain the efficacy profile of newly synthezised azo species.

MATERIALS and METHODS

Synthesis of 2-(substituted phenyl)azo-4,6-diacetylresorcinol

Substituted aniline (0.001mol) was dissolved in 2ml HCl and to it was added 1ml of H_2O. The solution was cooled to 0-5˚C in an ice bath and maintained this temperature. Sodium nitrite (0.002mol) in water (2ml) was then added drop wise. Stirring was continued for 20minutes to produce diazoniun salt at the same temperature. To this mixture, 4,6-diacetyl resorcinol (0.001mol, 0.194g) dissolved in 10% NaOH was added drop wise with stirring at 0-5˚C. The mixture was stirred for 15minutes. The precipitated crude azo product was collected by filtration at vacuum and recrystallised from appropriate solvent.[8] The structure of the six azo derivatives were represented in figure 1.

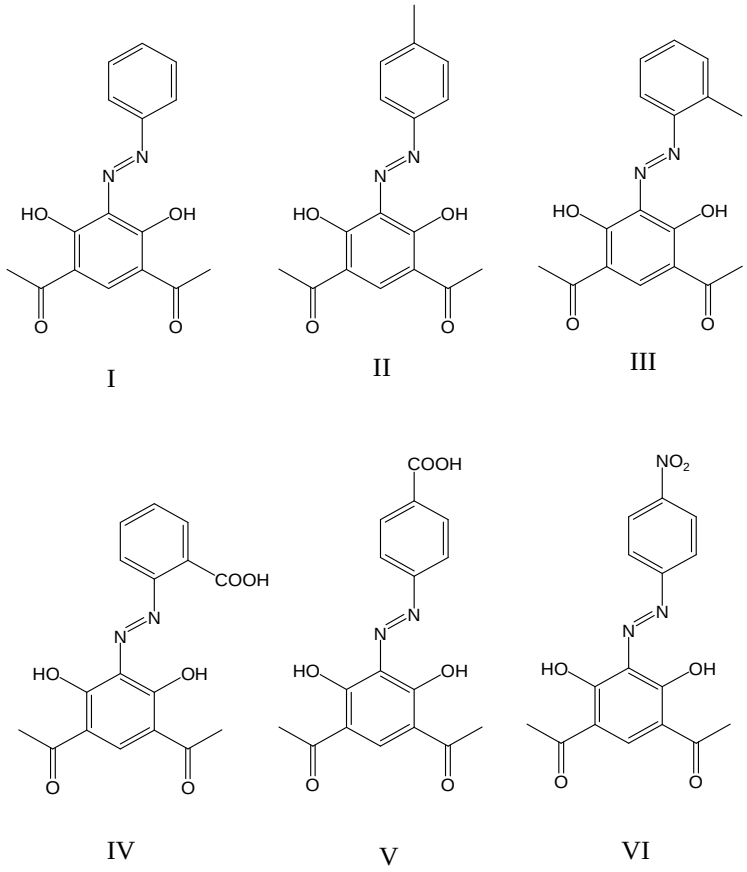

Figure 1: Structures of compounds I-VI

Molecular docking studies

Protein structure preparation

The X-ray crystallographic structure of the Epidermal growth factor receptor (PDB ID: 2ITO) was obtained from Protein Data Bank.[9] As a rule, the protein was prepared using the Protein Preparation Wizard. Preprocessed bond orders were assigned, hydrogens were added, metals were treated, water molecules were deleted and co- crystal ligand was removed from the crystal structure. Protein energy minimization was carried out until the average RMSD 0.30Å.[10]

Ligand structure preparation

The six synthesized azo compounds were drawn using Chemdraw.[11] Then these compounds were further imported into ligprep for ligand preparation. LigPrep involves series of steps that can produce a number of structures from each input structure with various ionization states, tautomers, stereochemistries, and ring conformations, and eliminate molecules using various criteria including molecular weight or specified numbers and types of functional groups

present. The default options in the LigPrep panel remove unwanted molecules, add hydrogens, and minimize the ligand structure.[12]

Docking and scoring function

Docking simplifies the amount of time spent for the library screening at the same time, efficient method which operates with algorithm includes several quantum mechanical parameters. In this present study, a top notch docking performer, glide version 5.5 was employed for molecular docking studies. Protein – ligand is a step by step methodology, which starts from protein and ligand molecule preparation, grid generation and actual docking process. Since the crystal structure of target protein has a co-crystal ligand, Grid generation can be restricted to that region alone for further assessment of unknown ligands. Hence, six synthesized ligand molecules were docked into active site of the receptor using glide extra precision method (XP).[13] Docking score, hydrogen bond interactions, hydrophobic interactions were analyzed by the use of glide XP visualizer.

ADME prediction

The key criteria which determine the successful transmission of just a lead molecule to drug like molecule is when they possess necessary absorption, distribution, metabolism and excretion properties to trigger a biological act. ADME properties were predicted using QikProp v3. 7. It predicts both physicochemically significant descriptors and pharmacokinetically relevant properties. All the analogs were neutralized before being used by QikProp.[14] The predicted properties were reported in Table 2.

RESULTS and DISCUSSION

Binding Mode Analysis Of Compound I With EGFR

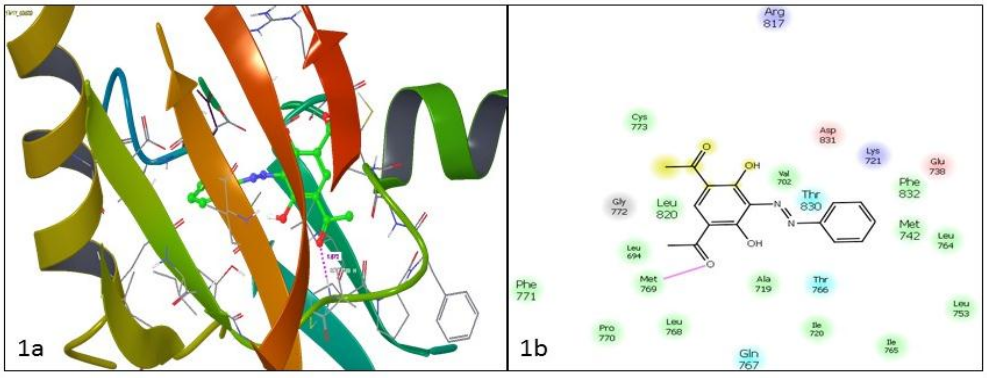

Figure 2: (a) 3D docked structure of compound I and EGFR, (b) 2D docked structure of compound I and EGFR.

The binding conformation of compound I (Figure 2) within the active site of the EGFR has been clearly shown in 3D docked structure. Upon the examination of docking features between compound I and EGFR it is found that there is only one hydrogen bond interaction. The back bone hydrogen atom of the hydrophobic residue Met 769 is interacted with the oxygen atom of carbonyl group of the compound I with a bond length 1.94Å. The docking studies further revealed that Cys 773, Val 702, Leu 820, Phe 832, Met 742, Leu 764, Ala 719, Ile720, Ile 765, Leu 694, Leu 820, Phe 771 and Pro 770 residues are involved in hydrophobic interactions. The 2D and 3D Docked structures of the target protein EGFR between compound I shows that the compound I is well interacted with EGFR and has good affinity towards the active pocket, thus, it may be considered as a good antagonist of EGFR. The glide score and glide energy values of the docked structure are -6.274 kcal/mol and -43.626 kcal/mol respectively. The glide score and glide energy of six azo compounds were reported in table1. The docking pose of the six azo compounds with EGFR were depicted in figure 2-7.

Binding mode analysis of compound II with EGFR

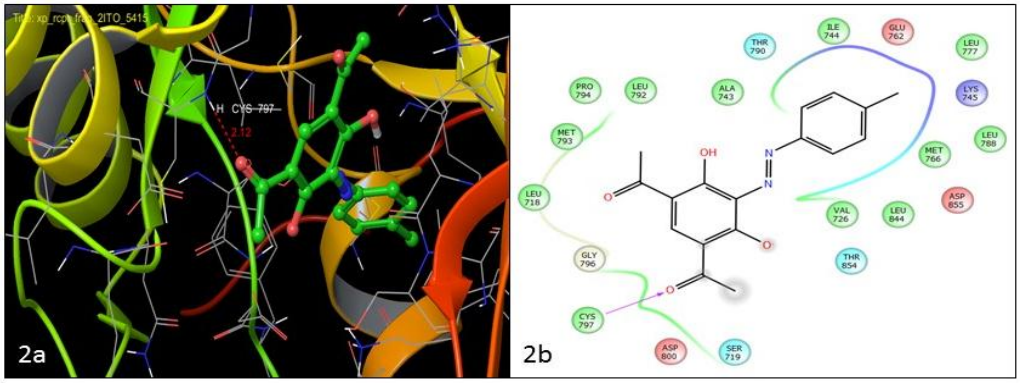

Figure 3: (a) 3D docked structure of compound II and EGFR, (b) 2D docked structure of compound II and EGFR.

The binding intensity of the compound II within the binding cleft of the EGFR was analyzed. Backbone hydrogen atom of the hydrophobic residue Cys 797 was well interacted with the oxygen atom of the compound II with a bond length 2.12Å. The glide score and glide energy was calculated and it was -5.180 kcal/mol and -40.812 kcal/mol respectively.

Binding mode analysis of compound III with EGFR

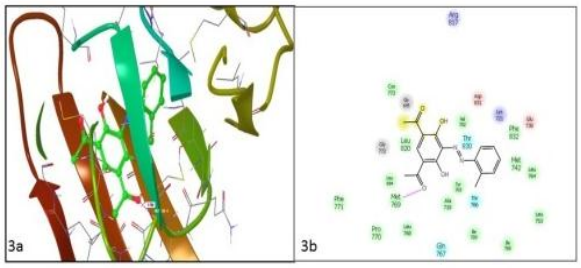

Figure 4: (a) 3D docked structure of compound III and EGFR, (b) 2D docked structure of compound III and EGFR.

The binding intensity of the compound III within the binding cleft of the EGFR was analyzed. After analyzing docking pose of the compound III with EGFR, one hydrogen bond interaction was noticed between hydrogen atom of compound III and back bone hydrogen atom of met 769 with bond length 1.79Å. The glide score and glide energy was calculated and it was -7.994kcal/mol and -42.802kcal/mol respectively.

Binding mode analysis of compound IV with EGFR

The binding propensity of the compound IV within the binding site of the EGFR was analyzed. After analyzing aggressive bonding of the compound IV with EGFR, two hydrogen bond interactions were occurred. Among that, backbone hydrogen atom of the Met 793 was strongly interacted with oxygen atom of the compound IV with bond length 2.04Å, followed by that backbone hydrogen atom of the Cys 797 was perfectively interacted with oxygen atom of the compound IV with bond distance of 2.05Å. The glide score and glide energy was calculated and it was -5.457kcal/mol and -50.802kcal/mol respectively. Amazingly, a salt bridge interaction was observed between polar residue Lys 745 and compound IV.

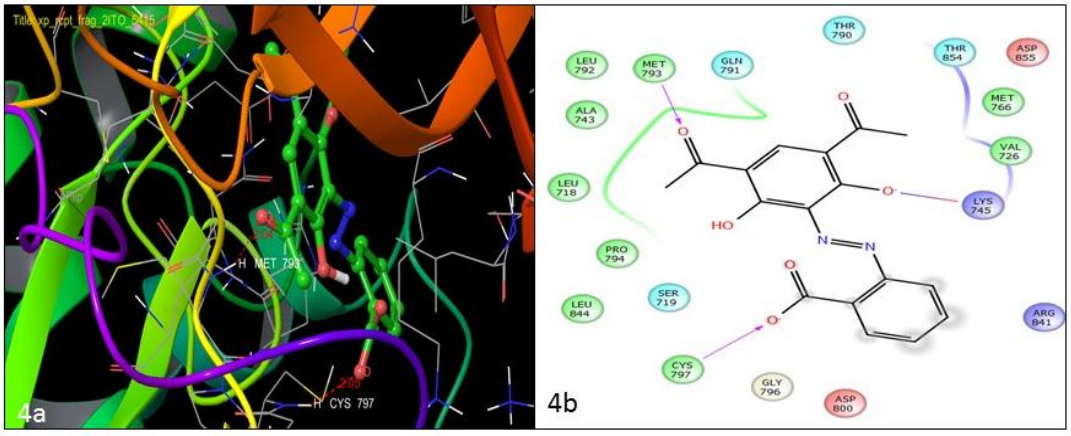

Figure 5: (a) 3D docked structure of compound IV and EGFR, (b) 2D docked structure of compound IV and EGFR.

Binding mode analysis of compound V with EGFR

The molecular assessment of the compound V with the target EGFR was analyzed. The investigation of bonded and non-bonded interactions yielded four hydrogen bond interactions involving the amino acids namely Met 793, Lys 728, Lys 716. The most captivating interaction of the compound V is the formed salt bridge interactions with Lys 728 and Lys 716 respectively. The glide score and glide energy was calculated and it was -5.867kcal/mol and -48.091kcal/mol respectively.

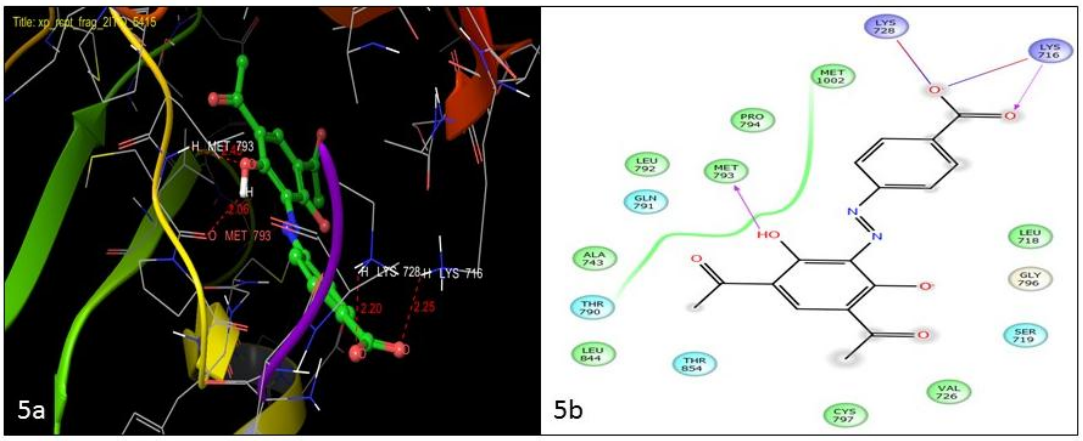

Figure 6: (a) 3D docked structure of compound V and EGFR, (b) 2D docked structure of compound V and EGFR.

Binding mode analysis of compound VI with EGFR

The critical bonding that may further implicate compound VI as good antagonist was two hydrogen bond interactions formed in the binding pocket of EGFR. In the cleft, two hydrogen bond interactions includes hydrogen atom of the compound VI formed a with side chain oxygen atom of the Asp 855 and backbone hydrogen atom of Phe723 interacted with the oxygen atom of compound VI. Surprisingly, a salt bridge and π- stacking interactions were observed. The glide score and glide energy was calculated and it was -5.099kcal/mol and -53.205 kcal/mol respectively.

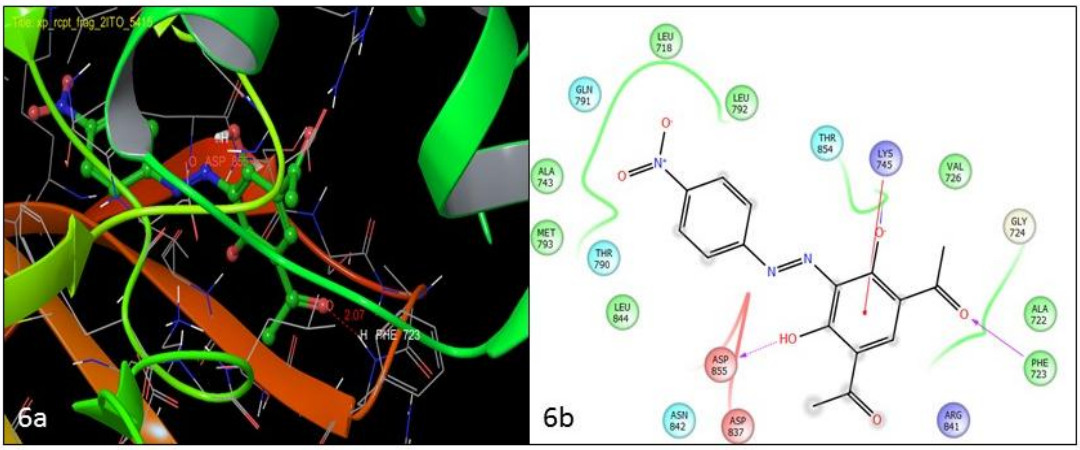

Figure 7: (a) 3D docked structure of compound VI and EGFR, (b) 2D docked structure of compound VI and EGFR.

Table1: Docking results of EGFR with azo compounds of 4, 6-diacetylresorcinol (I-VI).

Compound	Glide Score	Glide Energy	N. of H bond interactions	Interacting Residues	Distance (Å)
I	-6.274	-43.626	1	MET 769	1.94
II	-5.180	-40.812	1	CYS 797	2.12
III	-7.994	-42.802	1	MET 769 H	1.79
IV	-5.457	-50.967	2	MET 793 CYS797	2.04 2.05
V	-5.867	-48.091	4	MET 793 (2) LYS 728 LYS 716	2.45 2.06 2.20 2.25
VI	-5.099	-53.205	2	ASP 855 PHE 723	1.79 2.07

Table2: ADME results based on rule of five of azo compounds of 4, 6-diacetylresorcinol (I-VI).

Compound	Lipinski's rule of five (RO5)				QPLog S	Log BB
	Number of H bond donors	Number of H bond acceptors	MW	log P for octanol/water		
I	0.0	5.5	298	2.037	-3.267	-1.521
II	0.0	5.5	312	2.358	-3.838	-1.566
III	0.0	5.5	312	2.410	-3.620	-1.377
IV	1.0	7.5	342	1.679	-3.362	-2.321
V	1.0	7.5	342	1.544	-3.634	-2.692
VI	0.0	6.5	343	1.382	-3.586	-2.701

Molecular Weight (under 500 Daltons).

Number of H bond donors (0 to 1).

Number of H bond acceptors (5.5 to 7.5).

QP log BB for brain/blood (QPLogB/B) (-3.0 / 1.2).

Predicted aqueous solubility (acceptable range (26.5 to 0.5).

Predicted octanol/water partition co-efficient log p (acceptable range: 22.0 to 6.5).

ADME Properties Prediction

We have analyzed a new series of azo compounds of 4,6-diacetylresorcinol(I-VI) using QikProp v3.7 tool of Schrodinger software. The ADME values of newly synthesized compounds (I-VI) are given in Table 2. The first five properties are based on Lipinski rule of five, molecular weight (mol_MW) less than 500, partition coefficient between octanol and water (logPo/w) between 1.382 and 2.410, number of hydrogen bond donors between 0 and 1, number of hydrogen bond acceptors between 5.5 and 7.5 and aqueous solubility (logS) between -3.267 and -3.838, Brain/blood partition coefficient (logBB) between -1.377 and -2.701 indicated about the ability of the drug to pass through the blood–brain barrier which is mandatory for inhibition of EGFR kinase. All synthesized compounds (I-VI) obeyed Lipinski's rule of five and showed ADME properties in acceptable range.

CONCLUSION

EGFR is one of the specific targets approached in bladder cancer. This study aimed to unlock the therapeutic potential of six azo derivatives 4,6-diacetylresorcinol. Computational tools such as molecular docking and *insilico* pharmacokinetics prediction is employed to know the significance of synthesized molecules. All the synthesized azo compounds of 4,6-diacetylresorcinl (I-VI) have minimum binding energy, good affinity towards the active pocket and furthermore values of ADME are highly promising which warrants the therapeutic use of six azo derivatives.

ACKNOWLEDGEMENT

The authors are thankful to the Principal and the Management committee members of Jamal Mohamed College (Autonomous) for providing laboratory facilities. One of the authors (KL) is grateful to UGC-SERO, Hyderabad for financial assistance.

REFERENCES

1. Cancer Facts & Figures, American Cancer Society, 2015.
2. A. Ullrich, L. Coussens, J.S. Hayflick, T.J. Dull, A. Gray, A.W. Tam, J. Lee, Y. Yarden, T.A. Libermann, J. Schlessinger, J. Downward, E.L.V. Mayes, N. Whittle, M.D. Waterfield, P.H. Seeburg, Human epidermal growth factor receptor cDNA sequence and

aberrant expression of the amplified gene in A431 epidermoid carcinoma cells, Nature., 1984; 309: 418–425.

3. L.J. Slieker, T.M. Martensen, M.D. Lane, Synthesis of epidermal growth factor receptor in human A431 cells. Glycosylation-dependent acquisition of ligand binding activity occurs post-translationally in the endoplasmic reticulum, J. Biol. Chem., 1986; 261: 15233–15241.
4. Holbro T, Hynes NE, ErbB receptors: directing key signaling networks throughout life, Annu. Rev. Pharmacol. Toxicol., 2004; 44: 195–217.
5. G. Carpenter, S. Cohen, Epidermal growth factor, J. Biol. Chem. 1990; 265 7709–7712.
6. A. Wells, EGF receptor, Int J. Biochem. Cell. Biol., 1999; 31: 637–643.
7. Y. Yarden, M.X. Sliwkowski, Untangling the ErbB signalling network, Nat. Rev. Mol. Cell. Biol., 2001; 2: 127–137.
8. K. Loganathan, K. Sithick Ali, M. Purushothaman, S. Silambarasan and A. Jamal Abdul Nasser, J. Chem. Pharm. Res., 2015; 7(4): 1452-1455.
9. Yun CH, Boggon TJ, Li Y, Woo MS, Greulich H, Meyerson M, Eck MJ, Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. Cancer Cell., 2007; 11(3): 217-27.
10. Protein preparation Wizard Maestro (2013) New York: Schrodinger LLC.
11. http://www.cambridgesoft.com.
12. Ligprep, Version 2.7, Schrodinger, LLC, New York, NY, 2013.
13. Glide, Version 6.0 (2013) Schrodinger, LLC, New York, NY.
14. QikProp, Version 3.7, LLC, New York, NY, 2013.